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FEDERALLY OWNED TREATMENT WORKS (FOTW) DEMONSTRATION TEST

CHRONIC AQUATIC TOXICITY OF HD BIOEFFLUENTS

Mark V. Haley
Carl W. Kurnas

RESEARCH AND TECHNOLOGY DIRECTORATE

Steven D. Turley
Dennis T. Burton

WYE RESEARCH AND EDUCATION CENTER
Queenstown, MD 21658

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The U.S. Army Alternative Technology Program, Aberdeen Proving Ground, MD, has investigated an alternative method for disposing of sulfur mustard [2,2'-dichlorodiethyl sulfide (HD)], a chemical warfare agent under the chemical stockpile disposal program. The mineralization of HD, through hot water hydrolysis followed by biodegradation in sequencing batch bioreactors, has been demonstrated to be an effective technology. In this report, the feasibility of discharging effluents, produced from the bioreactors, to a Federally Owned Treatment Works (FOTW) has been investigated. The chronic toxicity of bioreactor effluent and effluents produced from an FOTW model were evaluated using the fathead minnow (*Pimephales promelas*) and daphnid (*Ceriodaphnia dubia*). The results from this research indicate that the discharge of bioeffluent to a wastewater treatment plant will not be toxic to ceriodaphnia and fathead minnows.

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PREFACE

The work described in this report was authorized under Sales Order No. 8J1W5A, Alternative Technology Program. This work was started in January 1996 and completed in December 1997.

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CHRONIC AQUATIC TOXICITY OF HD BIOEFFLUENTS

1. INTRODUCTION

Congress has mandated that all chemical agent stockpiles are to be destroyed by the year 2004. This mandate has stimulated efforts by the U.S. Army to develop alternate technologies to incineration that are safe and environmentally friendly.

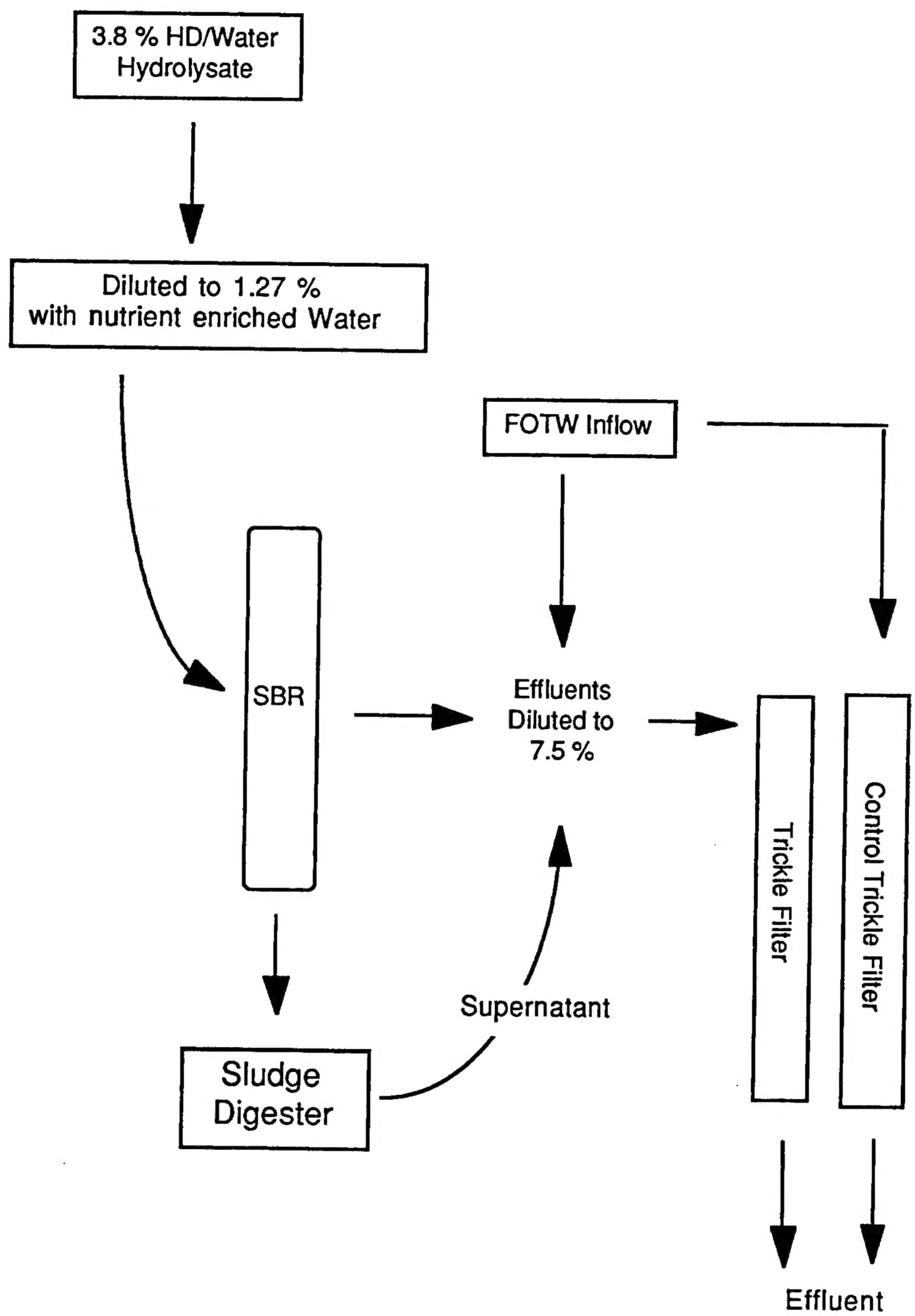
The U.S. Army's Alternative Technology Program [Aberdeen Proving Ground (APG), MD] has developed a neutralization/biodegradation process that treats hydrolyzed sulfur mustard [2,2'-dichlorodiethyl sulfide (HD)].[1] Hydrolyzed mustard is fed into sequencing batch bioreactors (SBR) that were seeded with biomass from a local wastewater treatment facility. The reactors were able to reduce the total organic carbon levels by 90-95%. We propose that effluents produced by the bioreactors be discharged into the wastewater treatment facility (WWTF) located at APG.

The National Research Council has determined that neutralization/ biodegradation exhibited the best process for meeting the U.S. Army's needs for disposing of HD. The U.S. Army has adopted neutralization/biodegradation as a proven technology for destroying mustard and is moving forward for state approval.

The U.S. Environmental Protection Agency (EPA) requires any discharge into waters of the United States be monitored through Whole Effluent Toxicity (WET) testing.[2] The primary purpose of WET testing is to ensure that wastewater discharges do not adversely affect aquatic life. WET testing is a series of chronic assays that monitor survival, growth, and reproduction as end point evaluations. WET testing is typically used to support municipal and industrial wastewater discharge permitting. These tests estimate the toxicity of discharges using the whole effluent approach, thereby measuring possible toxic interactions of various components in the discharge streams.

When a new waste is added to the process stream, the state requires additional chronic toxicity testing be performed above the standard testing frequency. If the effluent is toxic, extensive research is required to determine where the toxicity is originating.

To determine if adding SBR effluents to the WWTF would be toxic, a laboratory-scale demonstration test was conducted. A set of trickling filters (control and test) were designed and built by SBR Technologies, Incorporated (Southbend, IN)[3] to model the WWTF located at the Edgewood area (EA), APG, MD. The trickling filters were seeded with biomass from the WWTF. The control trickle filter received the wastewater feed stream from the WWTF. The treatment trickle filter received the wastewater feed stream plus 7.5% SBR effluent. The figure herein illustrates the design of the laboratory-scale demonstration test. Chronic toxicity bioassays were conducted using the fathead minnow (*Pimephales promelas*) and daphnid (*Ceriodaphnia dubia*). The results from these studies will be used to determine if discharging the SBR effluents to the WWTF will cause toxicity at the end of the discharge pipe.



Note: The trickle filters were designed to mimic the FOTW.

Figure. Design and Flow of the Laboratory-Scale Demonstration Test

2. MATERIALS/METHODS

The HD samples used for the neutralization (hydrolysis) reactions were taken from 1 ton storage containers. The HD (3.8%) was reacted with 90 °C water to produce thiodiglycol and hydrochloric acid as the major hydrolysis by-products (hydrolysate). The pH of the hydrolysate was adjusted using NaOH. Then the hydrolysate was diluted to 1.27% and fed to the SBR. After the ton containers (TCs) were drained, a solid material referred to as the heel remained. The heel was removed and dissolved/hydrolyzed using the same procedure described above. The hydrolyzed heel material will be referred to as TC cleanout hydrolysate (TCCH).

There were two types of feed streams used during this study. The first feed contained 1.27% hydrolysate without TCCH. After toxicity testing was completed, the bioreactor was slowly acclimated to the second feed. The second feed consisted of 1.27% hydrolysate containing TCCH [The initial 3.8% hydrolysate (consisting of 91% HD and 9% TCCH) was diluted to 1.27%]. The effluent produced from the SBR, which contained TCCH, was also subjected to WET testing.

The design case that the full-scale pilot plant will be running is that of the SBR runs with TCCH in the feed stream. Any regulatory decisions should be based on the results of testing with TCCH in the feed stream. The SBR was run with and without TCCH to determine if adding TCCH would disrupt the reactors and cause a drastic change in toxicity.

All aquatic toxicity testing was based on EPA Protocol No. 600-4-91-002, "*Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Water to Freshwater Organisms.*"[4] Fish studies were conducted at the U.S. Army Edgewood Research, Development and Engineering Center (ERDEC). The ceriodaphnia studies were conducted at the University of Maryland's Wye Research and Education Center (Queenstown, MD). These studies were conducted under good laboratory practices and conformed to all interagency standard operating procedures.

Effluent samples were taken on Fridays, Mondays, and Wednesdays and used within 6 hr of sampling. Samples taken on Fridays were used up to 48 hr. All other samples were used up to 24 hr. All samples were stored/transported at 4 °C. The EPA protocol allows sample use up to 72 hr as long as the initial use was within 36 hr.

2.1 Ceriodaphnia Assays.

The *Ceriodaphnia dubia* were obtained from the Philadelphia Academy of Natural Sciences (Philadelphia, PA). The ceriodaphnia were grown in media consisting of 20% Perrier Mineral Water and 80% water after reverse osmosis [RO (20/80 water)]. Perrier and RO water were mixed and aerated over night to drive out the carbonation and raise the pH. The organisms were maintained as batch cultures in 800 mL of media (20/80 water). The batch cultures were maintained for 14 days while initiating new cultures every 5-7 days. Ceriodaphnia were fed a mixture of *Selenastrum capricornutum* (green algae) and cerophyl. The algae were grown in EPA media for approximately 7 days before being harvested then fed to the ceriodaphnia at a concentration of 10^6 cells/mL. The cerophyl was prepared by suspending 5 g of cerophyl in 1 L of distilled water. The mixture was placed on a stirring plate and stirred overnight. The suspended material was filtered through a coffee filter to remove the large

particulate. Cerophyl stock was added to the media to yield a concentration of 4 mg of dry solids/liter. The additions of algae and cerophyl were done just before the ceriodaphnia were transferred to new media.

Approximately 2 weeks before testing, 25 adults were isolated from the batch cultures for offspring production. The second brood produced was grown to adults for producing offspring (either F3 generation or higher <24 hr old) to be used in testing.

All glassware used for testing and culturing was washed with nonphosphate soap, rinsed with tap water until sudsing had ceased, rinsed twice with distilled water, and heated to approximately 465 °C for 3 hr.

The test chambers consisted of 30-mL glass beakers using 15 mL of solution. There were 10 replicates for each treatment group (100, 75, 50, 25, 12.5, and 6.3%) and control containing one ceriodaphnia. The media was changed and fresh food added daily. Mortality, reproduction, pH, and dissolved oxygen were recorded every 24 hr. The light cycle was maintained at 16 hr light/8 hr dark. The light intensity was approximately 90 ft-c. The room temperature was maintained at 25 °C.

2.2 Fish Assays.

Fathead minnows used in testing were produced from in-house bred stock. Adult fish were originally purchased from Kurt's Fish Hatchery (Elverson, PA.) Every 12 months, new stock was purchased to renew the gene pool.

Water for the fish assays was drawn from a 375-ft deep well located next to Building E-3224 at EA-APG. The water was passed through an in-line air injector system (micronizer), pH buffer tank (dolomite bed), iron removal system, activated charcoal filters, particulate filters, and a UV sterilizer. Well water was analyzed semiannually by the National Testing Laboratories, Incorporated (Ypsilanti, MI) for 96 different ground water contaminants ranging from heavy metals to pesticides.

The adult fish were maintained in 55-gal glass aquariums equipped with under-gravel filtration units. The adults were fed twice daily [once with *Lumbriculus variegatus* (black worms) in the mornings and once with Tetramin Flake Food in the afternoons]. Water temperature was maintained at 25 °C with a light/dark cycle of 16/8 hr.

Terra-cotta flower pots were placed in the tanks to provide egg laying substrates. The pots were checked every morning for eggs. If eggs were present, the pots were removed and placed into a flow through tank. Eggs were checked daily for fungal growth and cleaned if needed. Incubation time was approximately 6-7 days. At the start of hatching, the eggs were placed in a static container. The fish larvae were collected within 24 hr of hatching and used in testing.

The test chambers consisted of 1-L glass jars with screw top lids that were scrubbed with phosphate-free soap, rinsed with tap water, then rinsed with distilled water. Effluent was added to the test chambers and diluted with well water to the desired concentration (100, 75, 50, 25, 12.5, or 6.2%) and allowed to stand until the temperature equilibrated. The test chambers were assigned table positions using the MiniTab computer program for random number generation.[5] The test was conducted at 25 °C with a day/night light cycle of 16/8 hr.

The larvae were gently placed into test chambers using a large bore plastic pipette and were fed freshly hatched brine shrimp twice daily (no feeding was done on day 7), once in the mornings and once in the afternoons following water changes. The water changes were done by siphoning out the water and debris using an air tube and a 1-mL plastic pipette. Any larvae accidentally siphoned out were transferred back and noted. Approximately 10% of the solution remained in the jars to allow the larvae room to swim freely.

Dissolved oxygen, pH, and mortality were recorded daily up to 7 days. At the end of 7 days, the larvae were removed from the test chambers, rinsed with distilled water, and dried in an oven at 100 °C for 2 hr. The dry weights of the larvae were measured to the nearest 0.01 mg using a Sartorius R200D Electronic Balance (Sartorius GmbH, Goettingen, Germany).

2.3 Statistical Evaluation.

Statistical evaluation was conducted on survival, reproduction, and growth after 7 days. Reproduction, growth, and minnow survival data were subjected to Shapiro-Wilk's test for normality and Bartlett's test for homogeneity. If either test failed, the data was transformed and retested for normality and homogeneity. If the data failed after transformation, a non-parametric test (Steel's Many-one Rank Test) was performed to determine the no observable effects concentration (NOEC) and the lowest observable effects concentration (LOEC). If the data passed tests for normality and homogeneity, the Dunnett's test was used to determine the NOEC and LOEC. Ceriodaphnia survival data were subjected to the Fisher's Exact test to determine if there were any significant survival differences. The EC_{50} (concentration that effects 50% of the organisms) was calculated using the Trimmed Spearman Karber method. All the statistical calculations were completed using computer software by WEST Incorporated (Cheyenne, WY).[6]

The IC_{25} (concentration that causes a 25% reduction in reproduction or growth) was determined using the Norberg-King method.[7] The IC_{25} was used for determining chronic toxicity of effluents based on instream waste concentration (IWC).

3. RESULTS

The SBR effluent without TCCH material was slightly more toxic to ceriodaphnia than was the SBR effluent with TCCH material. The fathead minnow showed the SBR effluent without TCCH material to be slightly less toxic than SBR effluent with TCCH material. Over all, the ceriodaphnia were more sensitive to the effluents than the fathead minnows.

3.1 Fathead Minnow.

Exposure to SBR effluent (produced from 1.27% feed without TCCH) for 7 days significantly decreased fathead minnow survival. Concentrations up to 25% did not affect survival. The 7-day EC_{50} was 37.5% effluent by volume. The NOEC and LOEC for survival were 25 and 50% effluent by volume, respectively. Exposure to effluent concentrations as high as 25% effluent by volume had no effects on growth. However, at a concentration of 50% effluent by volume, growth was significantly reduced. The NOEC and LOEC for growth were 25 and 50% effluent by volume (Tables 1 and 2).

Table 1. NOEC and LOEC Values for Fathead Minnows*

	Survival		Growth	
	NOEC (%)	LOEC (%)	NOEC (%)	LOEC(%)
SBR Effluent (1.27% Feed)	25	50	25	50
SBR Effluent (1.27% Feed w/TCCH)	25	50	12.5	25
Trickle Filter Effluent (1.27% Feed)	100	NE	100	NE
Trickle Filter Effluent (1.27% Feed w/TCCH)	100	NE	100	NE

NE - No effects were seen (up to 100%)
NOEC - No Observable Effects Concentration
LOEC - Lowest Observable Effects Concentration

**The data presented in this table represent the effects of SBR and trickling filter effluents (with and without TCCH) on the survival and growth of fathead minnows.*

Table 2. Raw Data for Fathead Minnow Exposure to SBR Effluent Without TCCH*

Concentration (% vol/vol)	% Survival (7-Day Exposure)	Growth (mg dry weight)
50	5	0.220
25	92.6	0.493
12.5	93.5	0.540
6.2	92.8	0.492
3.1	100	0.437
Control	97.4	0.521
Survival: NOEC - 25% LOEC - 50%		Growth: NOEC - 25% LOEC - 50%

	95% Confidence Intervals
48-hr EC_{50} > 50%	No Confidence Limits
7-Day EC_{50} = 37.5%	35.6 - 39.4%
7-Day IC_{25} (Growth) = 30.6%	27.0 - 31.9%

**The data presented in this table represent fathead minnow survival and growth when exposed to SBR effluent produced from 1.27% feed without TCCH.*

Exposure to SBR effluent (produced from 1.27% feed with TCCH) for 7 days also significantly decreased fathead minnow survival. Concentrations up to 25% did not affect survival. The 7-day EC_{50} was 34.7% effluent by volume. The NOEC and LOEC for survival were 25 and 50% effluent by volume, respectively. Exposure to effluent concentrations as high as 12.5% effluent by volume had no effects on growth. However, at a concentration of 25% effluent by volume, growth was significantly reduced. The NOEC and LOEC for growth were 12.5 and 25% effluent by volume, respectively (Tables 1 and 3).

Table 3. Raw Data for Fathead Minnow Exposure to SBR Effluent with TCCH*

Concentration (% vol/vol)	% Survival (7-Day Exposure)	Growth (mg dry weight)
50	0	—
25	90.0	0.334
12.5	90.0	0.529
6.2	100	0.548
3.1	100	0.569
Control	100	0.503
Survival: NOEC - 25%, LOEC - 50%	Growth: NOEC - 12.5%, LOEC - 25%	

	95% Confidence Intervals
48-hr EC_{50} > 50%	No Confidence Limits
7-Day EC_{50} = 34.7%	32.4 - 36.9%
7-Day IC_{25} (Growth) = 20.4%	18.7 - 21.5%

**The data presented in this table represent fathead minnow survival and growth when exposed to SBR effluent produced from 1.27% feed with TCCH.*

Exposure to trickling filter effluent (produced from SBR effluent with and without TCCH) for 7 days did not significantly affect either survival or reproduction. The NOEC for survival and growth was 100% effluent by volume (Tables 1, 4, and 5).

Table 4. Raw Data for Fathead Minnow Exposure to Trickling Filter Effluent Without TCCH*

Concentration (% vol/vol)	% Survival (7-Day Exposure)	Growth (mg dry weight)
100	97.4	0.538
50	95	0.586
25	100	0.621
12.5	100	0.511
6.2	97.5	0.511
Control	97.5	0.488
Survival: NOEC - 100%	Growth: NOEC - 100%	

	95% Confidence Intervals
48-hr EC_{50} > 100%	No Confidence Limits
7-Day EC_{50} > 100%	No Confidence Limits
7-Day IC_{25} (Growth) > 100%	No Confidence Limits

**The data presented in this table represent fathead minnow survival and growth when exposed to trickling filter effluent produced from 1.27% SBR effluent without TCCH.*

Table 5. Raw Data for Fathead Minnow Exposure to Trickling Filter Effluent with TCCH*

Concentration (% vol/vol)	% Survival (7-Day Exposure)	Growth (mg dry weight)
100	90	0.391
50	93.3	0.490
25	93.3	0.463
12.5	96.6	0.444
6.2	100	0.437
Control	100	0.425
Survival: NOEC - 100%		Growth: NOEC - 100%

	95% Confidence Intervals
48-hr EC_{50} > 100%	No Confidence Limits
7-Day EC_{50} > 100%	No Confidence Limits
7-Day IC_{25} (Growth) > 100%	No Confidence Limits

**The data presented in this table represent fathead minnow survival and growth when exposed to trickling filter effluent produced from 1.27% SBR effluent with TCCH.*

3.2 Ceriodaphnia.

Exposure to SBR effluent (produced from 1.27% feed without TCCH) for 7 days significantly decreased ceriodaphnia survival. Concentrations up to 9% did not affect survival. The 7-day EC_{50} was 10.6% effluent by volume. The NOEC and LOEC for survival were 9 and 12.5% effluent by volume, respectively. Exposure to effluent concentrations as high as 3% effluent by volume had no affects on reproduction. However, at a concentration of 6% effluent by volume, reproduction was significantly reduced. The NOEC and LOEC for reproduction were 3 and 6% effluent by volume, respectively (Tables 6 and 7).

Table 6. NOEC and LOEC Values for Ceriodaphnia*

	Survival		Reproduction	
	NOEC (%)	**LOEC (%)**	**NOEC (%)**	**LOEC(%)**
SBR Effluent (1.27% Feed)	9	12.5	3	6
SBR Effluent (1.27% Feed w/TCCH)	12.5	25	6.2	12.5
Trickle Filter Effluent (1.27% Feed)	100	NE	50	100
Trickle Filter Effluent (1.27% Feed w/TCCH)	100	NE	50	100
Trickle Filter Effluent (Control)	100	NE	100	NE

NE - No effects were seen (up to 100%)
NOEC - No Observable Effects Concentration
LOEC - Lowest Observable Effects Concentration

**The data presented in this table represent the effects of SBR and trickling filter effluents (with and without TCCH) on the survival and reproduction of ceriodaphnia.*

Table 7. Raw Data for Ceriodaphnia Exposure to SBR Effluent Without TCCH*

Concentration (% vol/vol)	% Survival (7-Day Exposure)	Average No. of Young Produced
15	0	0
12	20	0
9	90	4.1
6	100	5.3
3	100	24.6
Control	100	25.9
Survival: NOEC - 9% LOEC - 12%	Reproduction: NOEC - 3% LOEC - 6%	

	95% Confidence Intervals
48-hr EC_{50} = 14.5%	No Confidence Limits
7-Day EC_{50} = 10.6%	9.6 - 11.6%
7-Day IC_{25} (Reproduction) = 3.8%	3.7 - 4.0%

The data presented in this table represent ceriodaphnia survival and reproduction when exposed to SBR effluent produced from 1.27% feed without TCCH.

Exposure to SBR effluent (produced from 1.27% feed with TCCH) for 7 days significantly decreased ceriodaphnia survival. Concentrations up to 12.5% did not affect survival. The 7-day EC_{50} (determined by the Trimmed Spearman Karber method) was 18.7% effluent by volume. The NOEC and LOEC for survival were 12.5 and 25% effluent by volume, respectively. Exposure to effluent concentrations as high as 6.2% effluent by volume had no effects on reproduction. However, at a concentration of 12.5% effluent by volume, reproduction was significantly reduced. The NOEC and LOEC for reproduction were 6.2 and 12.5% effluent by volume, respectively (Tables 6 and 8).

Table 8. Raw Data for Ceriodaphnia Exposure to SBR Effluent with TCCH*

Concentration (% vol/vol)	% Survival (7-Day Exposure)	Average No. of Young Produced
50	0	0
25	0	0
12.5	100	5.3
6.2	100	27.3
3.1	100	29.1
Control	100	27.2
Survival: NOEC - 12.5% LOEC - 25%	Reproduction: NOEC - 6.2% LOEC - 12.5%	

	95% Confidence Intervals
48-hr EC_{50} = 18.7%	No Confidence Limits
7-Day EC_{50} = 18.7%	No Confidence Limits
7-Day IC_{25} (Reproduction) = 8.0%	7.7 - 8.2%

The data presented in this table represent ceriodaphnia survival and reproduction when exposed to SBR effluent produced from 1.27% feed with TCCH.

Exposure to trickling filter effluent (produced from SBR effluent without TCCH) for 7 days had no affects on ceriodaphnia survival. The 100% treatment group had one individual die; however, this was not significant. The 7-day EC_{50} and the LOEC for survival could not be determined. The NOEC for survival was 100% trickling filter effluent by volume. Exposure to effluent concentrations as high as 50% effluent by volume had no affects on reproduction. However, at a concentration of 100% effluent by volume, reproduction was significantly reduced. The NOEC and LOEC for reproduction were 50 and 100% effluent by volume, respectively (Tables 6 and 9).

Table 9. Raw Data for Ceriodaphnia Exposure to Trickling Filter Effluent Without TCCH*

Concentration (% vol/vol)	% Survival (7-Day Exposure)	Average No. of Young Produced
100	90	3
50	100	23.3
25	100	27.1
12.5	100	24.8
6.2	100	25.6
Control	100	25.9
Survival: NOEC - 100%	Reproduction:	NOEC - 50% LOEC - 100%

	95% Confidence Intervals
48-hr EC_{50} > 100%	No Confidence Limits
7-Day EC_{50} > 100%	No Confidence Limits
7-Day IC_{25} (Reproduction) = 60.0%	54.6 - 62.7%

***The data presented in this table represent ceriodaphnia survival and reproduction when exposed to trickling filter effluent produced from 1.27% SBR effluent without TCCH.**

Exposure to trickling filter effluent (produced from SBR effluent with TCCH) for 7 days had no affects on ceriodaphnia survival. Concentrations up to 100% had no affects on survival. The 7-day EC_{50} and the LOEC for survival could not be determined. The NOEC for survival was 100% trickling filter effluent by volume. Exposure to effluent concentrations as high as 50% effluent by volume had no affects on reproduction. However, at a concentration of 100% effluent by volume, reproduction was significantly reduced. The NOEC and LOEC for reproduction were 50 and 100% effluent by volume, respectively (Tables 6 and 10).

Exposure to control trickling filter effluent (produced from the feed stream supplying the FOTW) for 7 days had no affects on survival and reproduction (up to and including 100% effluent by volume). The NOEL for survival and reproduction was 100% control trickling filter effluent by volume (Tables 6 and 11).

When reviewing this data, readers should pay close attention to the effluents containing TCCH material. When in full production, the design case will include TCCH as part of the feed stream to the SBRs.

Table 10. Raw Data for Ceriodaphnia Exposure to Trickling Filter Effluent with TCCH*

Concentration (% vol/vol)	% Survival (7-Day Exposure)	Average No. of Young Produced
100	100	0.5
50	100	25.8
25	100	27.9
12.5	100	27.2
6.2	100	28.0
Control	100	27.2
Survival: NOEC - 100%	Reproduction:	NOEC - 50% LOEC - 100%

	95% Confidence Intervals
48-hr EC_{50} > 100%	No Confidence Limits
7-Day EC_{50} > 100%	No Confidence Limits
7-Day IC_{25} (Reproduction) = 60.0%	56.7 - 62.6%

**The data presented in this table represent ceriodaphnia survival and reproduction when exposed to trickling filter effluent produced from 1.27% SBR effluent with TCCH.*

Table 11. Raw Data for Ceriodaphnia Exposure to Control Trickling Filter Effluent*

Concentration (% vol/vol)	% Survival (7-Day Exposure)	Average No. of Young Produced
100	100	26.0
50	100	26.1
25	100	26.3
12.5	100	26.7
6.2	100	26.6
Control	100	26.4
Survival: NOEC - 100%	Reproduction:	NOEC - 100%

	95% Confidence Intervals
48-hr EC_{50} > 100%	No Confidence Limits
7-Day EC_{50} > 100%	No Confidence Limits
7-Day IC_{25} > 100%	No Confidence Limits

**The data presented in this table represent ceriodaphnia survival and reproduction when exposed to control trickling filter effluent.*

4. DISCUSSION

The Maryland Department of the Environment (MDE) regulations state that an effluent is not acutely toxic if the 48-hr EC_{50} > 100% (there cannot be more than 50% of the test organisms affected when exposed to 100% effluent by volume). The 48-hr EC_{50} generated from animals exposed to trickling filter effluent was >100%. Therefore, laboratory demonstration design has shown that the effluent produced from the trickling filter was not acutely toxic to either ceriodaphnia or fathead minnows. The 48-hr EC_{50} generated from exposure to SBR

effluent was <100%, and therefore, by definition, was acutely toxic to ceriodaphnia and fathead minnows. Since the salt concentration of the SBR effluent was approximately 2%, the suspect cause of toxicity was dissolved solids.

The dissolved solids in the SBR effluent consisted primarily of sodium, chloride, and sulfate ions. Half of the dissolved solids in the effluent are a result of initial pH adjustment after HD hydrolysis. The remaining salts are generated by the biodegradation process and additional pH adjustments when needed. An issue of concern is whether either the salt or residual dissolved organics are causing the SBR effluent to be toxic to the test organisms. There have been attempts to separate the salt and organic components so the toxicity of each can be determined. However, these attempts have either added additional salts to the effluent or eliminated both the salts and organics. If we can determine that the dissolved solids are the primary cause of toxicity associated with the SBR effluent, the disposal of the effluent may be less restrictive.

Freshwater organisms are placed under significant osmotic stress in water with extremely high dissolved solids (salts). Even if the salinity is at acceptable levels, the ionic ratio of dissolved solids can play a critical role in the organisms' abilities to maintain proper osmotic balance and cellular regulation.[8,9] Research is currently being conducted, based on a paper by McCulloch and coworkers,[10] to address the issue of dissolved solid toxicity. These studies involve preparing a synthetic effluent containing only dissolved solids. The synthetic effluent will be diluted with SBR effluent, and the toxicity will be evaluated.

The MDE regulations state that an effluent is not chronically toxic when the IC_{25} (concentration that reduces either growth or reproduction 25%) is greater than the IWC.[11] The IWC was computed using the following equation:

$$IWC = [QD/(QD + QRW)] \times 100 \qquad (1)$$

where

QD = volume of bioeffluent
QRW = 30-day low flow average (Bush River) over 5 years

At the time this report was written, a QRW value for the Bush River could not be documented. However, the IWC for the Gunpowder Neck Wastewater Treatment Facility (GNWWTF) was listed with MDE as 3%. Assuming a maximum discharge of 1 MGD (QD) from the GNWWTF, the above equation can be solved for QRW, using equation 2.

$$QRW = [QD(100\text{-}IWC)] \times IWC \qquad (2)$$

The QRW for the Bush River was calculated to be 32.3 MGD. During full-scale pilot plant production, 0.075 MGD of bioeffluent will be produced. The effluent will be discharged to the GNWWTF to produce a combined volume of <1 MGD. Using equation 1, where the QD = 0.075 MGD and the QRW = 32.3 MGD, the IWC for the effluent produced from the SBRs would be 0.23%.

The IC_{25} for fathead minnows generated from the trickling filter effluent (with and without TCCH) was >100% effluent by volume. The IC_{25} for ceriodaphnia generated from the trickling filter effluents was 60% (with and without TCCH) (Tables 4, 5, 9, and 10). Therefore, the effluent produced from the trickling filters, by definition, was not chronically toxic to either ceriodaphnia or fathead minnows.

The IC_{25} for fathead minnows exposed to SBR effluent was 20.4% (with TCCH) and 30.6% (without TCCH). The IC_{25} for ceriodaphnia exposed to SBR effluent was 8% (with TCCH) and 3.8% (without TCCH). All the IC_{25} values for SBR effluent were greater than the IWC (0.23%). Therefore, by definition, the SBR effluent was not chronically toxic to either ceriodaphnia or fathead minnows.

Stream flow data obtained from the Maryland Geological Survey can also be used to estimate the flow of the bush river. However, caution should be taken when using the following data. Most of the data points are of 30-day, low flow averages over 10 years, and one data point is a 7-day average flow over 10 years. The MDE requires a 30-day, low flow average over 5 years. Therefore, the results generated from the following data may be suspect.

There are five streams that flow into the head of the Bush River above the GNWWTF. Table 12 lists the five streams (Bush River Basin) and flow data. Also listed in this table is the 7-day, low flow average over 2 years, which was included for comparison purposes only. The QRW for the Bush River was computed using the 10-year data to yield a conservative value. When the stream flows are added together and converted to MGD, the resulting QRW for the Bush River was 10.5 MGD. Using this value and 0.075 MGD of bioeffluent being discharged, the IWC was determined to be 0.7%. Even with such a conservative IWC value, the IC_{25}s were greater, and the bioeffluent was still not considered to be chronically toxic.

Ceriodaphnia were also exposed to effluent produced from a control trickling filter to determine if materials in the federally owned treatment works (FOTW) feed stream were adding additional toxicity to the trickling filter effluents. The data showed that there were no affects on either ceriodaphnia survival or reproduction after 7 days of exposure (Table 11). Therefore, the FOTW feed stream did not contribute to the toxicity of effluent produced by the trickling filter.

5. CONCLUSIONS

Because the sequencing batch bioreactor (SBR) runs containing ton container cleanout hydrolysate (TCCH) in the feed stream was the design case for the full-scale pilot plant operations and ceriodaphnia were the most sensitive species, any regulatory decisions should be based on ceriodaphnia results with TCCH in the feed stream.

The EC_{50} value for ceriodaphnia is 18.7% SBR effluent by volume. If the feed to the trickling filter contains concentrations of SBR effluent below 18.7%, then the trickling filter effluent should not be acutely toxic to either ceriodaphnia or fathead minnows.

The effluent produced by the trickling filter was neither acutely nor chronically toxic to either ceriodaphnia or fathead minnows. The 48-hr acute toxicity results were >100% effluent, and the IC_{25} is greater than the instream waste concentration (IWC) (IWC = 0.23%).

Research is currently being conducted to provide further insight into whether the salt is the primary cause of toxicity in the effluents.

Table 12. Stream Flow for the Bush River Basin

Bush River Basin	Stream Flow[1] (ft^3/s)	Stream Flow[2] (ft^3/s)
Winter Run	12 (30Q10)	18 (7Q2)
Swan Creek	0.6 (7Q10)	1.4 (7Q2)
Bynum Run	3.2 (30Q10)	5.3 (7Q2)
James Run	0.5 (30Q10)	1.1 (7Q2)
Grays Run	0.0 (30Q10)	0.1 (7Q2)
Total Flow	16.3	25.9

16.3 ft^3/s x 448.8 = 7,315.4 gal/min x 60 min/hr x 24 hr/day = 10,534,233.6 gal/day
= 10.5 MGD

IWC = QD/(QD + QRW) x 100

where

QD = bioeffluent flow (0.075 MGD)
QRW = flow of Bush River (10.5 MGD)

IWC = 0.7%

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